Amended Claims for PCT Application No. PCT/EP99/10297

Claims:

1. An isolated polypeptide comprising an amino acid sequence which has at least 75% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72 over its entire length.

2. The polypeptide as claimed in claim 1 comprising the amino acid sequence selected from the group consisting of: SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 and 72.

3. An isolated polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

4. An isolated polypeptide comprising a fragment of at least 7 consecutive amino acids of the polypeptide as claimed in any one of claims 1 to 3, wherein the fragment comprises an epitope.

5. The polypeptide of claim 4, wherein the fragment is immunogenic.

6. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide that has at least 75% identity to the amino acid sequence of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

7. An isolated polynucleotide comprising a nucleotide sequence that has at least 75% identity to a nucleotide sequence, encoding a polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 75% identity to that of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length; or a nucleotide sequence complementary to said isolated polynucleotide.

9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 over its entire length.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72.

11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70 or 72, obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69 or 71 or a fragment thereof.

13. An expression vector comprising an isolated polynucleotide according to any one of claims 6 - 12.

14. A recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 - 12.

15. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell.

16. A process for producing the polypeptide of claim 1 comprising the steps of culturing a host cell of claim 15 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

17. A process for expressing a polynucleotide of any one of claims 6 - 12 comprising transforming a host cell with an expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

18. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

19. The vaccine composition of claim 18, wherein the polypeptide has an amino acid sequence selected from the group consisting of: SEQ ID NO:42, 46, 48, 50, 52, 54, 56, 58, 60 and 62.

20. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically acceptable carrier.

21. The vaccine composition according to any one of claims 18-20, wherein said composition comprises at least one other *Bordetella pertussis* antigen.

22. An antibody immunospecific for the amino acid sequence of claim 1 or 2, the polypeptide of claim 3 or the fragment of claim 4 or 5.

23. A method of diagnosing a *Bordetella pertussis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

24. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 – 5 in the preparation of a medicament for use in generating an immune response in an animal.

25. Use of a composition comprising an immunologically effective amount of a polynucleotide as claimed in any one of claims 6 - 12 in the preparation of a medicament for use in generating an immune response in an animal.

26. A therapeutic composition useful in treating humans with *Bordetella pertussis* disease comprising at least one antibody directed against the polypeptide of claims 1 – 5 and a suitable pharmaceutical carrier.

27. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 6-12 or a polypeptide of claims 1-5.

28. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;

sequencing the *lcrD*-like gene;

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

if a virulence-associated member, sequencing the entire pathogenicity island, and

identifying genes within this sequence.

29. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.